COPULATION IN THE ACŒLOUS TURBELLARIAN POLYCHŒRUS CARMELENSIS[1]

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Attention has been directed recently to the primitive condition of the reproductive system of the Turbellaria Acœla, as compared with most of the Platyhelminthes, and to the occurrence of copulation in the group (Hyman, 1937). The Acœla are hermaphroditic. Most members of the group possess a copulatory mechanism including an extensible penis and a seminal receptacle, provided with one or more small tubes called "Mundstücke," the function of which is assumed to be the transport of sperm from the receptacle or bursa toward the eggs. In view of the presence of a copulatory mechanism, von Graff (1905) assumed that true copulation is of widespread occurrence in the group.

Gardiner (1898) had earlier concluded that in *Polychœrus caudatus* sperm transfer is accomplished by a process of hypodermic impregnation, in which the "mouth pieces" function as hypodermic organs. Bresslau (1933) and Hyman (1937) admit that hypodermic impregnation may be of common occurrence in the Acœla. We have found no specific account of this process other than that of Gardiner (1898). Only two descriptions of true copulation in the Acœla have come to our attention, that of Peebles (1915) for *Monochœrus lineatus* and that of Hyman (1937) for *Amphiscolops langerhansi*. Only in the latter case was the observation of living animals supplemented by the study of sectioned pairs. The study of both living and sectioned specimens of *Polychœrus carmelensis*, a form very closely similar to *P. caudatus*, has yielded no evidence of the occurrence of hypodermic impregnation. The evidence demonstrates conclusively that impregnation is regularly accomplished by means of true copulation, the details of the process differing considerably from that described by Hyman for *Amphiscolops langerhansi*.

During the summer months of 1936 and 1937 *Polychœrus carmelensis* were collected in great abundance from tide pools at Pescadero Point on Carmel Bay, California, and in smaller numbers from tide pools elsewhere in the vicinity. Examination of living and sectioned specimens soon revealed certain structural differences from the Atlantic

Coast *Polychœrus caudatus*, as described in the papers of Mark (1892), Verrill (1893), Gardiner (1895, 1898), and Löhner (1910). On the basis of these differences the Pacific Coast form was designated as a distinct species (1938).

A brief description of the reproductive system of *P. carmelensis* will be presented in order to facilitate an understanding of the copulatory process. Previously, the reproductive system has been discussed only with respect to certain points in which it differs from that of *P. caudatus* (1938).

The reproductive structures of *P. carmelensis*, in ventral view, are illustrated diagrammatically in text fig. 1. The female system includes a pair of gonads which appear to originate anteriorly in a median "Eilager" (*ei*), a short distance posterior to the statocyst (*st*). Extending posteriorly from the Eilager are two lateral ventral rows of oöcytes (*o*) which, a short distance below the level of the mouth, become embedded in the vitellaria (*vi*), the latter terminating at the level of the female aperture (♀). In the vitellaria the young oöcytes are located ventrally, and move dorsally as they mature. The female gonads are typical of the Acœla in their lack of specialized investing walls and of ducts to either the exterior or to the bursa seminalis. The external female aperture is roughly elliptical, about one-sixth the width of the body, enormously distensible. From this aperture there extends dorsally, to a distance of about two-thirds the thickness of the body in this region, a large dorso-ventrally flattened chamber, the vagina. The anterior wall of the vagina bears, at a position somewhat dorsal to its mid-point, a thick-walled pocket (*vp*) which is directed anteriorly, slanting slightly dorsally, and provided with a small lumen. The anterior end of the pocket is embedded in the dorsal part of the bursa seminalis (*bs*), its lumen opening directly into the bursa much in the manner in which Mark described the vagina of *P. caudatus* as communicating at its dorsal end with the naked bursa tissue. We find in the literature no mention of a structure in *P. caudatus* corresponding to the vaginal pocket of *P. carmelensis*. The muscular walls of the vagina and pocket are lined with a nucleated epithelium lacking cilia. These structures are shown, in sagittal section, in plate figures 1 and 2.

TEXT FIG. 1. Ventral view, showing arrangement of male and female reproductive systems. Composite drawing, with projector, from two whole mounts stained with Delafield's hæmatoxylin, the testis follicles largely diagrammatic. Magnification 35 ×. *bs*, bursa seminalis; *cf*, caudal filament; *cl*, caudal lobe; *e*, eye; *ei*, "Eilager"; ♀, external female aperture; ♂, external male aperture; *m*, mouth; *mp*, mouth piece of bursa; *o*, oöcyte on way from Eilager to vitellarium; *p*, penis bulb; *st*, statocyst; *t*, testis follicles; *vi*, vitellarium containing young oöcytes and mature eggs; *vd*, vas deferens; *vp*, vaginal pocket.

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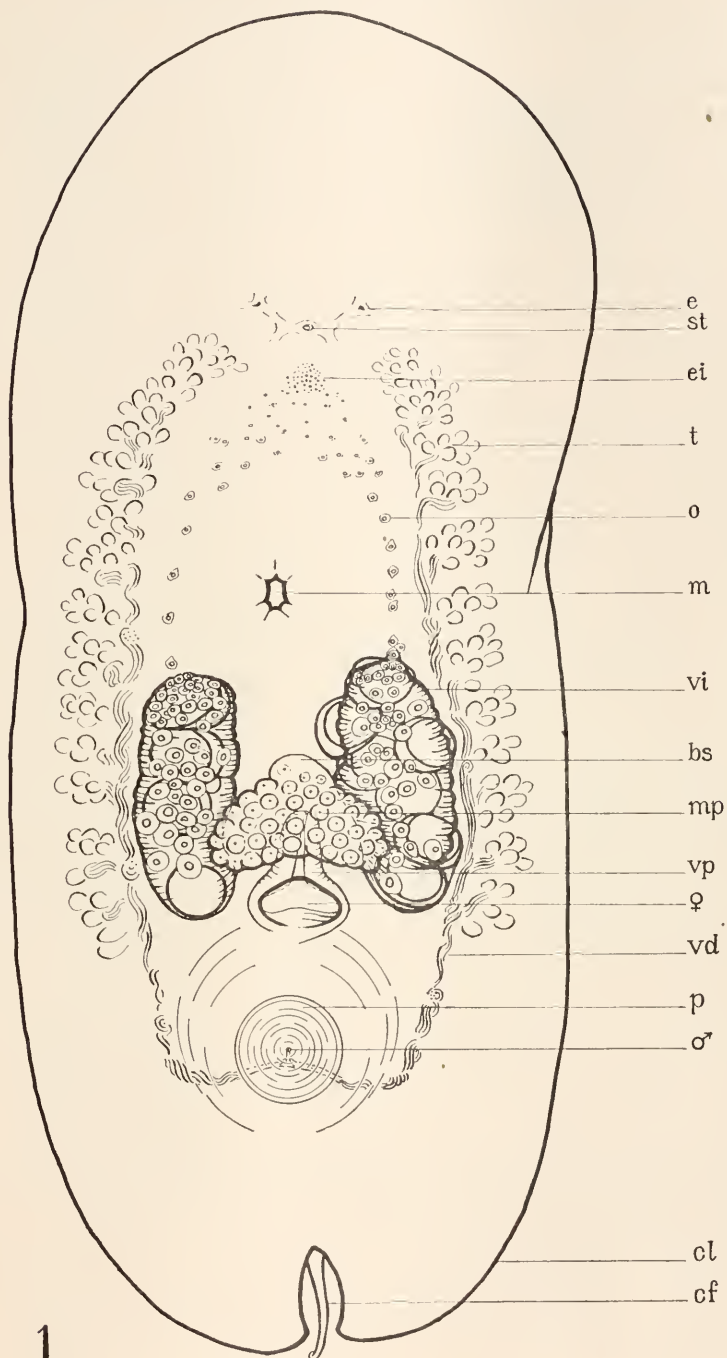
The bursa seminalis appears to resemble that of *P. caudatus* in all essential features. It is somewhat kidney-shaped, and is located medially between the two vitellaria, anterior to the female aperture and the vagina. It occupies the space between the vaginal pocket and the ventral body wall but extends anteriorly well beyond the pocket, the anterior dorsal portion surrounding the anterior tip of the pocket. The internal structure of the bursa is shown, in slightly oblique transverse section, in plate fig. 3. The entire ventral part of the bursa is composed of many small compartments, each containing a small tubular "mouth piece" (mp), the long axis of which approximately parallels the dorso-ventral axis of the body. The ventral small ends of these tubes open into the vacuolated marginal parenchyma (va), a thin layer of which separates the bursa from the ventral integument. At the basal end of each mouth piece is usually attached a mass of sperm (s'') occupying a small vacuole. The ventral compartments of the bursa merge dorsally into a chamber (bs') which frequently contains scattered sperm masses sometimes connected with the ventral masses.

Regarding the histology of the bursa a few facts may be mentioned. The syncytial tissue of which the bursa is composed sometimes appears almost continuous (plate fig. 3) except for the presence of sperm masses, muscle fibers and a few small vacuoles. The highly vacuolated appearance, especially of the dorsal part of the bursa, which seems to characterize many specimens (plate fig. 2), probably results largely from the disruptive influences of mating and egg-deposition, although the method of fixation is to some extent responsible, slowly penetrating fixatives permitting time for violent muscular contraction and consequent distortion of tissue. The bursa lacks a differentiated limiting wall. Ventrally, it impinges upon marginal parenchyma (in the terminology of Löhner); dorsally, it is bounded by the anterior pocket posteriorly and central parenchyma anteriorly (plate figs. 1 and 2); its anterior curved surface approximates the intestinal parenchyma (ip) from which it is separated by muscle fibers; laterally, it is enclosed by the vitellaria. The bursa communicates with the exterior by way
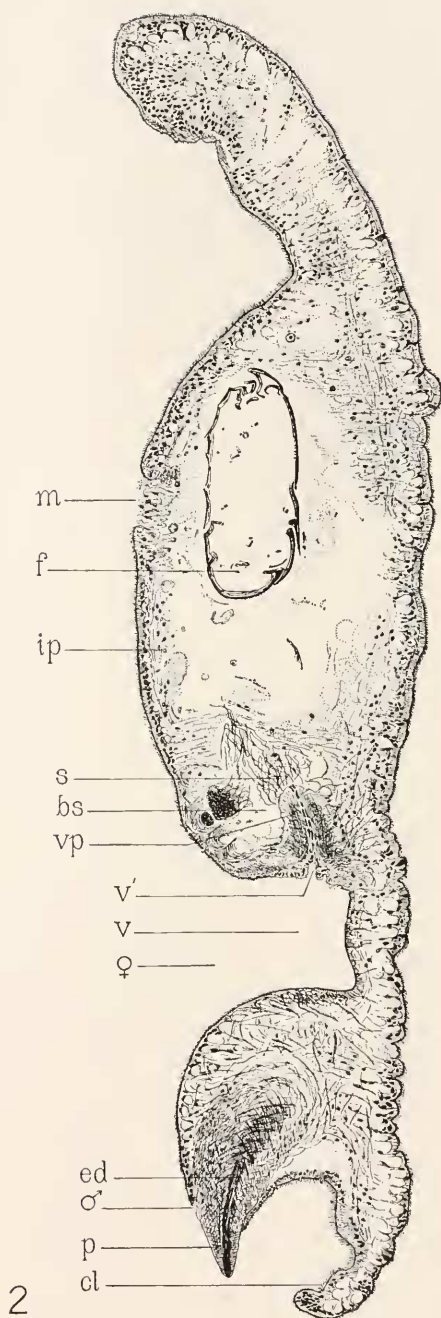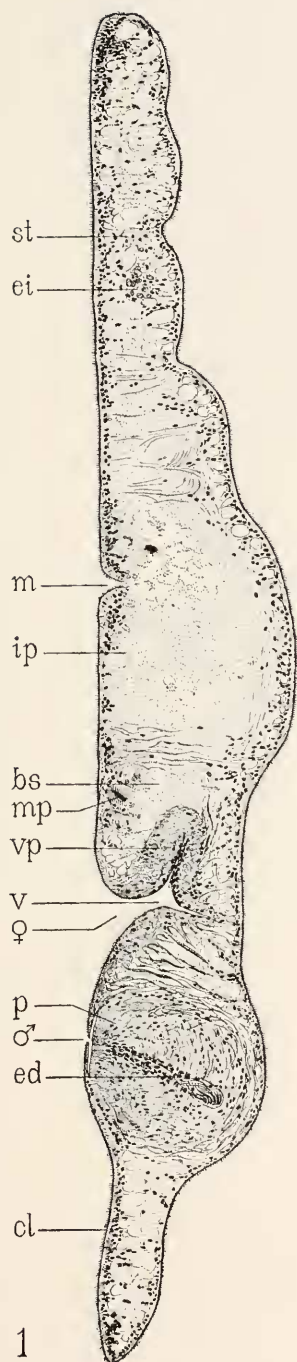
PLATE I

of the small lumen of the vaginal pocket, the vagina and the female aperture.

The male reproductive system (text fig. 1) includes a pair of gonads of follicular arrangement (*t*), extending from approximately the level of the statocyst to a point somewhat posterior to the female aperture. They are lateral and dorsal to the female gonads.   The vasa deferentia (*vd*) are a pair of channels in the parenchyma through which sperm pass from the follicles of each side to the ejaculatory duct (plate figs. 1 and 2, *ed*) of the penis (*p*).   The testes and vasa deferentia lack specialized investing walls; the ejaculatory duct is lined with a nucleated epithelium.   The penis is located posterior to the vagina.   It is compact, muscular; in the retracted state, an ovoid bulb, which occupies almost the entire thickness, and nearly one-fourth of the width, of the body in this region (plate fig. 1, *p*).   Its dorsal end is inclined somewhat posteriorly.   In the retracted state the ventral tip of the penis extends free into a small circular chamber which is covered by ventral integument bearing a very small, but greatly distensible, perforation, the male aperture ($\male$).   During activity the penis is protruded through the male pore, and assumes an elongated tapering conical form, arched so that the distal end is directed posteriorly (plate fig. 2, *p*).

The copulatory mechanism of *P. carmelensis* consists, in brief, of the male organ and aperture, the female aperture, the vagina with anterior pocket, and the bursa or seminal receptacle.

Material and Procedure

Collections of *Polychærus carmelensis* were brought into the laboratory on the *Ulva* (and a few other sea-weeds) upon which they are found in the tide pools.   Large numbers of individuals of all ages were removed immediately from the *Ulva* and preserved for later study. Usually a few hundred worms were distributed among several fingerbowls.   Most of the remainder of the *Ulva*, bearing thousands of worms and abundant food organisms (chiefly small crustacea), was placed into two running salt-water aquaria.   Here the worms remained alive and active for long periods of time.

During the summer of 1936 our attention was focused mostly on

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Plate II, Figure 3

Detailed portion of section of text fig. 3 (*a–b*), to show newly-acquired sperm mass (*s*) in the dorsal part of the bursa anterior to the vaginal pocket.   Drawn with aid of projector.   Magnification 180 ×.   *bs'*, surface of dorsal chamber of bursa; *o*, young oöcyte, and *o'*, nearly mature egg, in vitellarium; *s'*, sperm, apparently migrating from mass (*s*) toward ventral bursa mouth piece; *s''*, sperm ball attached to mouth piece; *va*, vacuole of ventral marginal parenchyma; other abbreviations as previously used.

PLATE II, FIGURE 3

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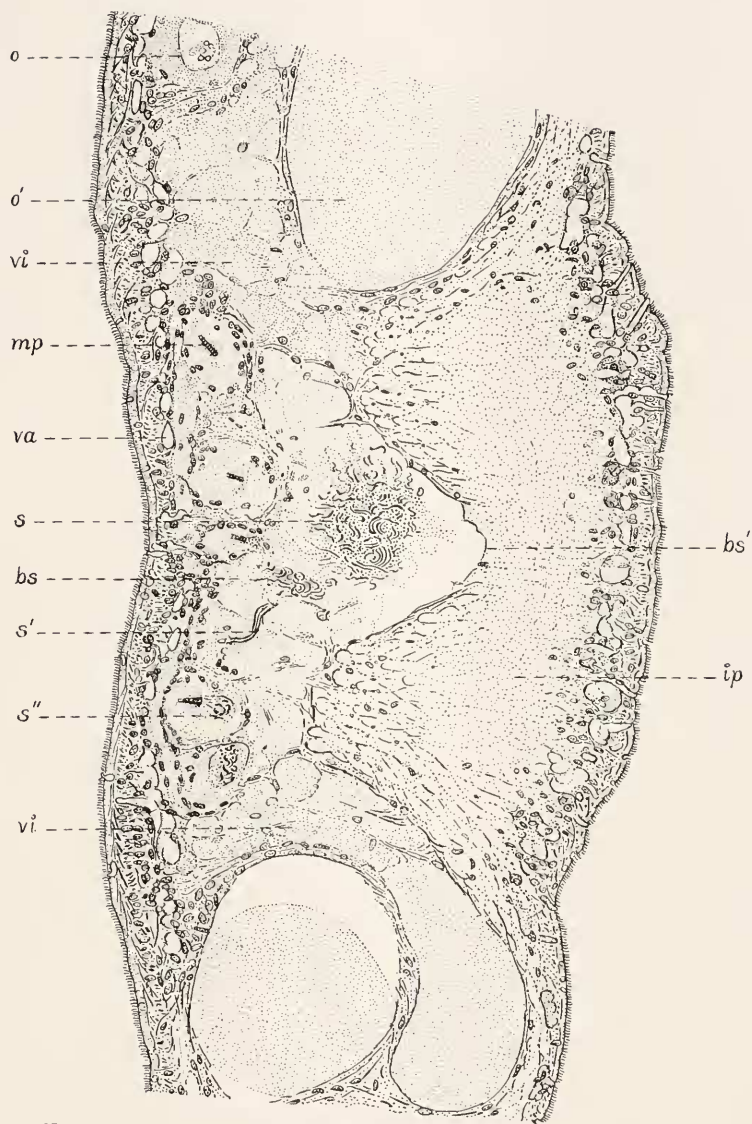
a study of the anatomy and development of *P. carmelensis*.   All observations of mating activities were made in 1937, in the period between June 24 and August 24.   Observations were rendered somewhat difficult by the extremely brief duration of mating; the entire process rarely exceeded a minute and a half.   In all, 35 matings were witnessed; most of these were observed in detail, usually with the aid of a hand lens.   Occasional pairs were removed to glass slides for immediate examination, and fifteen pairs were fixed for later sectioning and study. The pairs were transferred by means of a pipette from aquarium or fingerbowl to the fixative.   Unfortunately, the mates separated in every case, either during this transfer or at some time before the embedding process was reached.   This separation removed the possibility of subsequent identification of members of a single pair, since two or more pairs were usually stored in a single vial.   Only two pairs were stored separately.   However, the data furnish ample evidence with regard to the essential features of the mating process in *Polychœrus carmelensis*.

Observations

Copulation was most frequently observed on the glass sides of the aquaria where the worms were present in great numbers.   About one-third of the observed matings occurred in the fingerbowls.   Mating was never seen in the tide pools, but is assumed to have preceded the deposition of eggs which were found in abundance on small stones and in the folds of *Ulva*.   In *Polychœrus carmelensis* the eggs are fertilized before deposition, and there is no evidence of the occurrence, in this species, of self-fertilization or of any method of sperm transfer other than true copulation.

The 35 matings witnessed in the laboratory are listed in Table I, according to the respective dates and hours of occurrence.   The table shows that matings are infrequent during the late morning, afternoon, and evening, but are relatively numerous immediately after dawn, from 5 to 7 o'clock.   Periods of observation of the aquaria and fingerbowls included all hours of the day and night except from 1 to 4 A.M. However, in the table, hours of observation (+) are indicated, where records are sufficiently complete, only for days upon which one or more matings was witnessed.   While observations during the period from 4:45 to 9:30 A.M. were made only on the three dates listed, the observations during the usual working hours of the day, as well as at night, were far more extensive.   Therefore, the difference in frequency of mating in the early morning as compared with the later hours is probably greater than the total numbers indicate.   Indeed, after having repeatedly watched in vain for signs of mating, the occurrence

of nine or ten matings within 2 to $2\frac{1}{2}$ hours after dawn seemed a veritable epidemic. It is interesting to note that, whereas early morning is the preferred mating time, egg-deposition occurs usually after sundown.

There was considerable variation in the degree of activity of the worms during the month of observation, probably in some measure correlated with the length of time during which they had been kept in the laboratory. However, our data are of insufficient extent to provide evidence of this correlation.

The process of mating took place in the following manner (text fig. 2). Considerable restlessness was evidenced by certain mature-looking worms, which usually contained visible accumulations of sperm in the vasa deferentia and at the base of the ejaculatory duct.

TABLE I

The numbers of matings observed in the laboratory are listed according to the dates and hours of occurrence. The hours of observation (+) on the respective dates are indicated, where the records are sufficiently complete.

	4:45–5 A.M.	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–1 P.M.	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	Total observed matings
June 24........										1								1
July 28........						3												3
29........								1										1
31........	+	5	4	+	+													9
Aug. 2........						+	+	+				2					1	3
9........	+	4	3	3		3					+	+	1			1	+	15
13........							+	+		+	1	+						1
16........						+	+	+	+		1	+						1
24........		1	+	+						+	+	+	+					1
Total observed matings		10	7	3		6		1		1	2	2	1			1	1	35

Such individuals moved about actively, frequently elevating the head end, and pausing to exchange head contacts with other worms. In case such overtures between two worms were prolonged, the individuals faced each other with the anterior halves of their bodies touching ventrally and swaying slowly from side to side in opposite directions (2a). This process was sometimes terminated by one worm moving away. If continued, one animal presently bent its anterior half down under its partner, twisting at the same time so as to bring its ventral surface into contact with the ventral surface of its partner, and retaining attachment to the substratum along the posterior margin of one side. The partner at the same time arched dorsally, elevated the side adjoining the other individual, remaining attached only along the margin of one side (2b). The two worms then glided along each other in opposite directions, ventro-lateral margins in contact, until each

animal had passed more than half its length beyond its partner.   At this point, when the copulatory organs were presumably in juxta-position, one animal, with a sudden snap, released its attachment completely, both curled ventrally, the pair forming a ball attached only by the lateral marginal papillæ of one animal (2*c* and 2*d*).   After a connection of only 40 to 50 seconds, the attached animal uncurled, and began to move off, necessitating the reattachment of its partner. The entire mating process, including overtures, occupied only 1 to $1\frac{1}{2}$ minutes.

The mating position obviously conceals the actual relation of the genital regions of the two worms.   Mating pairs placed on slides for examination separated immediately.   However, they showed certain facts clearly.   The genital region invariably evidenced activity by
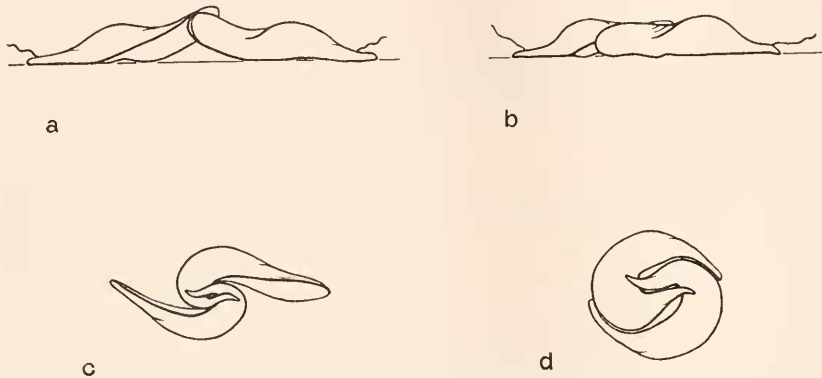

TEXT FIG. 2.   Series of sketches showing progressive stages in the assumption of the typical copulatory position.   *a*, *b*, mating pair viewed in plane parallel to the substratum; *c*, *d*, viewed in plane perpendicular to the substratum.

the protrusion of the penis, the repeated contraction and distention of the vagina and the female aperture.   In several individuals a large mass of sperm was visible in the dorsal part of the bursa anterior to the vaginal pocket.   Only once were sperm observed at the female aperture; there was never a trace of sperm in the vagina, pocket, or about the penis, though a fluid filled with oil-like globules issued from the penis of one individual.   The presence of sperm in the bursa, and their complete absence from vagina, pocket or penis, suggested that sperm might be deposited directly into the dorsal chamber of the bursa by the insertion of the penis of one worm into the lumen of the vaginal pocket of its mate.   Confirmatory and supplementary evidence was provided by the sectioned material.

A sagittal view of a typical individual fixed during copulation (plate fig. 2) shows striking differences from the typical non-copulant

(plate fig. 1). The body is usually curved ventrally though seldom retaining in fixation the full flexure of the mating position. Invariably, the penis (p) is protruded through the male aperture ($\male$), and the vagina (v) distended. The vaginal pocket (vp) is usually curved slightly ventrally, the epithelial lining of the lumen showing signs of activity in the antero-posterior orientation of the nuclei and the pseudopodial projections and occasional sloughing at the opening into the vagina. In the non-copulant the penis is retracted, the vagina and female aperture antero-posteriorly flattened. The pocket infre-
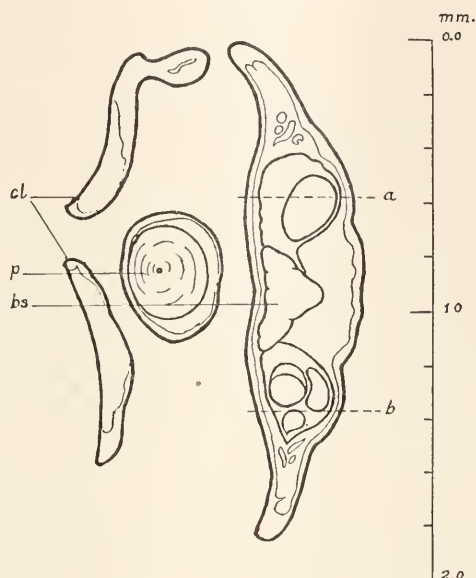
TEXT FIG. 3. Transverse section (slightly oblique dorso-ventrally) of individual fixed, during copulation, in Heath's solution and stained by the Flemming tri-color method. The ventral curvature typical of the copulatory position is shown by the presence of caudal lobes (cl), and penis (p), in section which passes through the anterior portion of the bursa (bs). Camera lucida drawing, with scale.

quently displays evidence of activity, and in some cases the lumen is not visible at the anterior end. A few individuals, such as that shown in text fig. 3, in transverse section, retained more completely the curvature of the typical copulatory position (text fig. 2, c and d). The body is bent ventrally at the level of the vagina, so that the transverse section through the anterior portion of the bursa (bs) passes also through the extended penis (p), and through the caudal lobes (cl). Thus, the copulatory organs of the fixed mating worms indicate activity and are in such a position as to permit the insertion of the

penis of each individual into the anteriorly-directed pocket of its mate.

The accomplishment of impregnation during copulation is evidenced by 16 of the sectioned worms. In these animals (plate figs. 2 and 3) a large mass of sperm (s) is found in the dorsal part of the bursa usually anterior to the pocket, but frequently connected by strands of sperm with the anterior end of the lumen. The absence of sperm elsewhere in the lumen, and in the vagina, in both fixed and living copulants, seems to justify the conclusion that the sperm are deposited directly into the dorsal chamber of the bursa by the insertion of the penis of the mate into the vaginal pocket, probably as far as the opening of the latter into the bursa. The large aggregates of sperm in the dorsal bursa are sometimes densely coiled (plate fig. 3), sometimes more scattered (plate fig. 2). They are embedded in the bursa tissue; several cases in which they appear to be embedded in the intestinal parenchyma probably represent distortion due to slow fixation. The enormous size of these sperm masses, their position anterior to the pocket, and their frequent connection with the pocket lumen, distinguish them from those found in random specimens. They are without doubt masses deposited during the mating which was terminated immediately before, or interrupted by, fixation. Three additional sectioned worms contained smaller masses of sperm which were judged to be newly-acquired. The remaining eleven worms gave no evidence of impregnation.

It is obvious that some of the fifteen pairs of worms were reciprocally impregnated since more than half of the individuals contained newly-acquired sperm. The two mating pairs which were preserved in separate vials were both cases of reciprocal impregnation. By noting for each vial in which more than one mating pair were preserved, the proportion of individuals containing newly-acquired sperm, it was found that at least six of the total fifteen matings involved reciprocal impregnation. Allowing for the disturbance of the mating worms incident to their transfer to the fixative, it appears probable that reciprocal impregnation is of more usual occurrence than the minimum figures indicate.

Thus, the results of examination of the fixed material are in harmony with, and supplement, the observations of the living mating worms. There was no evidence of the use of bursa mouth pieces for the accomplishment of sperm transfer by the hypodermic method, as postulated by Gardiner for *P. caudatus*.

The subsequent path of the sperm from the dorsal part of the bursa to the eggs will be considered only briefly at present. Undoubtedly,

some of the sperm migrate ventrally within the bursa to the mouth pieces, as evidenced by certain of the fixed copulants (plate fig. 3, *s'*). This is in accord with Mark's view of the distribution of sperm in the bursa of *P. caudatus*. We have as yet found no evidence of the passage of sperm through the mouth pieces to the eggs by way of the vacuolated ventral parenchyma, as Löhner postulated for *P. caudatus*. We hope to consider this subject more fully in a paper dealing with the maturation and fertilization of the eggs of *Polychœrus carmelensis*.

Discussion

Attention may be directed to certain similarities and differences in the copulatory process of the three species of Acœla for which it has been described, *Monochœrus lineatus*, *Amphiscolops langerhansi*, and *Polychœrus carmelensis*. The general copulatory position is similar in all cases in that the mates are united by the ventral surfaces of the posterior ends, their anterior ends extending in opposite directions. No further details of the process were given by Peebles (1915) for *Monochœrus lineatus*. In *Amphiscolops langerhansi* (Hyman, 1937), the mates assume the form of a ball for about thirty seconds prior to the assumption of the final mating position, in which they are unrolled and attached by their ventral surfaces. The ball form is closely similar to the final mating position characteristic of *P. carmelensis*. The prolonged union of forty to fifty-five minutes in *Amphiscolops* contrasts strikingly with the extremely brief union of forty to fifty seconds in *P. carmelensis*.

The relative positions of the reproductive organs of the mates differ, in the two species, in correspondence with structural differences. In *Amphiscolops*, the bursa opens into the female genital pore by way of a small invagination (Hyman, p. 322). The penis papilla is free only posteriorly, behind which there is a projecting sperm guide. Copulation occurs by the interlocking of penes. The sperm are directed toward the bursa of each individual by the sperm guide of the mate. Impregnation is mutual in *Amphiscolops langerhansi*.

Summary

The process of mating is described for *Polychœrus carmelensis*. A study of living and fixed mating individuals, separated from their mates, indicates that copulation involves the insertion of the penis of each individual into the vaginal pocket of the mate, so that sperm are deposited directly into the dorsal chamber of the seminal bursa. Impregnation is, in some cases, reciprocal. The entire process lasts only one to one and a half minutes.

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